Rejection under 35 USC §112, second paragraph

In paragraph 5 the Examiner made a series of suggestions for amendments to the claims in order to make the claim language more consistent and clear. Amendments were made in accordance with the suggestions made by the Examiner.

In response to the Examiner's suggestion in paragraph 5(e) to remove "derived from" from claims 3-5 Applicants would like to point out that the term "derived from" is defined in the specification at page 6 lines 19-28 and page 8 lines 18-29 so that the term would be sufficiently clear to one of skill in the art. Applicant's believe it is clear from the specification that nucleic acid that is isolated directly from an organism may serve as template to generate a nucleic acid sample, often an amplified sample, that is derived from the first sample by using the first sample as template.

Rejection under 35 USC §102

In paragraph 6 the Examiner rejected claims 45 and 46 as being anticipated by Hornes et al. (5,759,820) and in paragraph 7 the Examiner rejected claims 45-47 as being anticipated by Allan et al. (2001/0049094 A1). Applicants have canceled claims 45-49 making this rejection moot.

Rejection under 35 USC §103(a)

In paragraph 8 the Examiner rejected claims 1-16 in view of Alland et al. in view of Fisher et al. and further in view of Hornes et al. Claim 1 has been amended to add the limitation that the unwanted target sequence in the bait:target complex is digested (support for this amendment is found throughout the specification, for example in Figure 2). Alland et al. does not teach increasing the relative percentage of a population of interest by digestion of unwanted target sequences. Alland et al. teaches a method to separate unwanted clones from a library of cloned nucleic acids by hybridizing probes to unwanted sequences and detecting hybridization. Hybridization allows detection and identification of the clones containing unwanted nucleic acids, but Alland et al. does not teach a method of using the hybridized probe to digest the unwanted nucleic acids as is shown by the presently claimed invention. Alland also requires that each nucleic acid is

spatially separated from every other nucleic acid. In Alland et al. each nucleic acid is physically separated in microtiter plates and the unwanted clones that have been identified by hybridization are removed from the library by re-innoculating using all clones except those identified as unwanted clones. In the presently claimed invention the unwanted target sequences may be removed from a mixed population of nucleic acids without first physically separating each of the nucleic acids. Neither Fisher et al. nor Hornes et al. remedy the deficiencies of Alland et al. as neither teaches increasing the relative percentage of a population of nucleic acids of interest by digestion of unwanted target sequences.

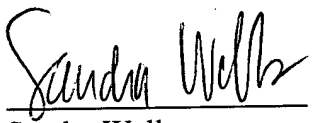
In paragraph 9 the Examiner rejected claims 17-44 and 49 over Alland et al. in view of Fisher et al. and Hornes et al. and further in view of Li et al. and Joyce et al. As indicated above Alland et al., Fisher et al. and Hornes et al. each fails to teach increasing the relative percentage of a population of interest by digestion of unwanted target sequences. Neither Li et al. nor Joyce et al. teach digestion of unwanted target sequences and enrichment of a population of nucleic acids of interest.

CONCLUSION

For the foregoing reasons, Applicants believe all the pending claims are now in condition for allowance and should be passed to issue. Applicants believe that no extension of time is required for submission of this paper. However, if an extension is required, Applicants petition for any necessary extension of time and authorize the Commissioner to deduct any required fees from the undersigned's Deposit Account No. 01-0431. Please deduct any additional fees from, or credit any overpayment to the above-noted Deposit Account. If the Examiner feels that a telephone conference would in any way expedite the prosecution of the application, please do not hesitate to call the undersigned at (408) 731-5768.

Dated: December 5, 2002

Respectfully submitted,



Sandra Wells
Reg. No. 52,349

Legal Department
Affymetrix, Inc.
3380 Central Expressway
Santa Clara, CA 95051
Tel: 408/731-5768 Fax: 408/731-5392

Version with markings to show changes

In the specification:

Paragraph beginning on page 20 line 20 with the following paragraph:

The following procedure was performed in PCR tubes in a thermocycler. An initial mixture was prepared by mixing 25 µ[:]g of total *E. coli* RNA to 13.75 µ[:]L of 5.0 µ[:]M rRNA Reverse Transcriptase (RT) Primer Mix, and adding deionized water (DI H_2O) to a final volume of 30 :L and a concentration of .83 µ[:]g/ µ[:]L of RNA.

Paragraph beginning on page 20 line 24:

The following primers were used to target 16S and 23S RNA (each primer is 5 µ[:]M in the RT primer mix):

16S1514 5'-CCTACGGTTACCTTGTT-3'
16S889 5'-TTAACCTTGCGGCCGTACTC-3'
16S541 5'-TCGATTAACGCTTGCACCC-3'
23S2878 5'-CCTCACGGTTCATTAGT-3'
23SEco2064 5'-CTATAGTAAAGGTTCACGGG-3'
23SEco1519 5'-TCGTCATCACGCCTCAGCCT-3'
23S1012 5'-TCCCACATCGTTTCCCAC-3'
23S539 5'-CCATTATACAAAAGGTAC-3'

Paragraph beginning on page 21 line 7:

To the above mixture, a reverse transcription mixture including 10 µ[:]L of 10X MMLV RT Buffer, 5 µ[:]L of 100mM DTT, 2:L of 25mM dNTP Mix, 3 µ[:]L of 24.5U/ µ[:]L RNAse Inhibitor (RNAguard Ribonuclease Inhibitor (Porcine), Amersham Pharmacia Biotech, P/N 27-0816-01), 6 µ[:]L 50U/ µ[:]g MMLV Reverse Transcriptase (Epicentre Technologies, P/N MCR85101) and 44 µ[:]L of DI H_2O was added and the

reaction was carried out at 42°C for 25 minutes and transferred to 45°C for an additional 20 minutes. The mixture was then transferred to 4°C.

Paragraph beginning on page 21 line 14:

The rRNA in the DNA:RNA hybrids was then digested by adding 5 μ[:]L of 10U/ μ[:]L RNAse H (Epicentre Technologies, P/N R0601K) at 37 C for 45 minutes. The enzyme was heat deactivated at 65°C for 5 minutes and then transferred to 4°C.

Paragraph beginning on page 21 line 17:

The DNA was then removed by adding 2.5 μ[:]L of 5U/ul DNAse I (Amersham-Pharmacia Biotech P/N 27-0514-01) and 1 μ[:]L of 24.5U/ μ[:]L RNAse inhibitor. Digestion was carried out at 37°C for 20 minutes and the enzyme was deactivated by adding EDTA to a final concentration of 10mM.

Paragraph beginning on page 21 line 28:

The removal efficiency for 16s and 23s rRNA is typically between 80-90%. Figures 6 and 7 show [shows] the results of hybridization of enriched and non-enriched RNA to microarrays. Fig. 6 shows hybridization of labeled unenriched RNA to a microarray. Fig. 7 shows hybridization of labeled enriched RNA to an identical microarray. As can be seen by comparing Figs. 6 and 7, the hybridization in Fig 7 shows a much cleaner hybridization with less signal produced by cross hybridization.

Paragraph beginning on page 22 line 8:

Cloned DNAs encoding the *E. coli* 16S and 23S rRNA genes were amplified separately by PCR and purified with the QIAquick PCR purification kit (QIAGEN P/N 28104). One μ[:]g of 16S and 1 μ[:]g of 23S rDNA were combined in a PCR tube and diluted to 25 μ[:]L with DI H_2O. The DNA was denatured by heating at 99°C for 5

minutes in a thermocycler. The tube was transferred to 70°C followed by the addition of 25 μ[:]L of a prewarmed (at 70°C) solution containing 1 μ[:]g *E. coli* total RNA, 200 mM NaCl, 100 mM Tris (pH 7.5). The tube was incubated at 70°C for 30 minutes to permit annealing of the rRNAs to the corresponding complementary strand of rDNA (approximately 1:1 molar ratio). The tube was then transferred to 37°C followed by the addition of 50 μ[:]L of a prewarmed (at 37 C) solution containing 2 units of *E. coli* RNAseH (Epicentre Technologies P/N R0601K), 50mM Tris (pH 7.5), 100mM NaCl, 20mM $MgCl_2$, and the reaction was incubated at 37°C for 20 minutes to digest RNA from DNA:RNA hybrids. DNA was then digested by the addition of 2 units of DNAse I (Epicentre Technologies, P/N D9902K) and incubation at 37°C for 15 minutes. EDTA was then added to a final concentration of 20 mM to inhibit further nuclease activity. RNA was purified with an RNeasy column (QIAGEN P/N 74104) and then analyzed in a denaturing agarose gel stained with ethidium bromide.

Paragraph beginning on page 23 line 4:

Cloned DNAs encoding the *E. coli* 16S and 23S rRNA genes were amplified separately by PCR and purified with the QIAquick PCR purification kit (QIAGEN P/N 28104). 0.6 μ[:]g of 16S and 0.6 μ[:]g of 23S rDNA were combined in a PCR tube and diluted to 48 μ[:]L with DI H_2O. The DNA was denatured by heating at 99°C for 5 minutes in a thermocycler. The temperature was lowered to 70°C followed by the addition of 48 μ[:]L of a prewarmed (at 70°C) solution containing 6 μ[:]g *E. coli* total RNA, 200 mM NaCl, 100 mM Tris (pH 7.5), and 12 units of thermostable RNAse H (Epicentre Technologies, P/N H39100). The tube was incubated at 70°C for 1 minute to permit annealing of the rRNAs to the corresponding complementary strand of rDNA (approximately 1 mole DNA per 10 moles RNA). The temperature was reduced to 50°C for 5 minutes to complete one cycle of enrichment. The temperature was then increased to 70°C for 1 minute then again reduced to 50°C for 5 minutes to complete the second cycle. This temperature cycling was repeated a total of 30 times. After 1, 5, 10, 20, and 30 cycles 16 μ[:]L (corresponding to 1 μ[:]g RNA from the starting mixture) was removed from the tube and mixed with 1 unit DNAse I (Epicentre Technologies, P/N

D9902K) and incubated at 37°C for 15 minutes. EDTA was then added to a final concentration of 20 mM to inhibit further nuclease activity. RNA was purified from each sample with an RNeasy column (QIAGEN P/N 74104) and then analyzed in a denaturing agarose gel, along with 1 μ[:]g of untreated *E. coli* total RNA (Figure 9). The diminishing amounts of 23S and 16S RNA as cycles are repeated can be seen by comparing the lanes from left to right. The first lane (labeled U) is untreated. The next lanes are the amount of 23S and 16S RNA after 1, 5, 10, 20 and 30 cycles, respectively.

In the claims

1. A method of preparing labeled fragments of a population of nucleic acids of interest [a nucleic acid] comprising:

increasing the relative percentage of [a] said population of nucleic acids of interest within a mixed population of nucleic acids, wherein said mixed population [of interest] comprises a plurality of nucleic acid sequences, comprising:

(a) contacting a nucleic acid sample with a nucleic acid bait molecule, wherein said nucleic acid sample comprises said nucleic acids of interest and at least one unwanted target sequence and wherein said bait molecule binds [is capable of complexing] specifically to [a] said unwanted target sequence, but not to said [sequences] nucleic acids [in said population] of interest, under such conditions as to allow for the formation of a bait:target complex;

(b) digesting the unwanted target sequence in the [removing said] bait:target complex [from said mixed population] thereby resulting in an increase in the relative percentage of said [population] nucleic acids of interest within said mixed population of nucleic acids;

fragmenting [the sequences from] said nucleic acids [population] of interest to produce fragments; and

adding a label [signal moiety] to the fragments.

6. The method of claim 1 wherein said [population of] nucleic acids of interest [is] are messenger RNA (mRNA[.]).

7. The method of claim 1 wherein said unwanted target sequence is [stable RNA] rRNA or tRNA.

9. The method of claim 1 wherein said unwanted target sequence is 23S RNA.

10. The method of claim 1 wherein said unwanted target sequence is 16S RNA.

11. The method of claim 1 wherein said bait molecule is generated from a population of nucleic acids other than the nucleic acids of interest [exogenously].

14. The method of claim 1 wherein said bait molecule is synthesized by hybridizing a primer to said unwanted RNA target sequence and extending said primer by reverse transcriptase using said target sequence as a template.

15. The method of claim 1 wherein the nucleic acid sample is an [and] RNA sample, the bait molecule is DNA, and the bait:target complex is a DNA:RNA hybrid.

17. The method of claim 1 wherein said bait molecule is attached to a solid substrate.

20. The method of claim 1 wherein said bait molecule is modified to comprise a selectable element.

22. The method of claim 20 further comprising the step of exposing said bait:target complex to a reagent that binds [capable of binding] said selectable element to form a reagent:bait:target complex prior to the step of digesting the unwanted target sequence.

23. The method of claim 22 wherein the reagent that binds [capable of binding] said selectable element is selected from the group consisting of: a nucleic acid sequence, a ligand, a receptor, an antibody, a haptenic group, an antigen, an enzyme or an enzyme inhibitor.

25. The method of claim 22 wherein said selectable element is biotin and said reagent that binds [capable of binding] said selectable element is streptavidin [streptavadin].

26. The method of claim 22 further comprising [wherein said step of removing said RNA sequence is accomplished by] separating said reagent:bait:target complex from said mixed population prior to the step of digesting the unwanted target sequence..

29. The method of claim 15 [28] wherein the step of digesting said unwanted target sequence comprises exposing said DNA:RNA hybrid to a reagent which digests RNA in a DNA:RNA hybrid and digesting said unwanted target sequence and wherein said reagent is RNAse H.

31. The method of claim 1 further comprising the step of removing the bait molecule after digesting the target sequence in said bait: target complex [wherein the step of removing said bait:target complex is a two step process in which the target is removed first and the bait molecule is removed thereafter].

32. The method of claim 29 further comprising the step of removing any remaining DNA bait molecules after said unwanted target [RNA] sequence is removed.

33. The method of claim 32 wherein said step of removing said DNA bait [molecule] molecules is accomplished by digestion with DNAse I.

38. The method of claim 1 wherein said label [signal moiety] is a biotin.

39. The method of claim 1 wherein said label [signal moiety] is a polyethylene oxide [PEO]-Iodoacetyl Biotin.

40. The method of claim 1 wherein the label [signal moiety] is attached to the 5' ends of said fragments.

41. The method of claim 1 [40] wherein after said step of fragmenting, said 5' ends of said fragments are chemically modified.

42. The method of claim 41 wherein the 5' ends of said fragments are chemically modified by γ[(]-S-ATP and T4 kinase.

43. The method of claim 41 [40] wherein said chemical modification results in the addition of a thiol group to the 5' end of said fragments.

44. The method of claim 43 wherein said detectable signal moiety is polyethylene oxide [PEO]-Iodoacetyl Biotin.